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1: Biochemistry. 1994 May 17;33(19):5838-45.

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**Expression and ligand binding characterization of the beta-subunit (p75) ectodomain of the interleukin-2 receptor.****Sana TR, Wu Z, Smith KA, Ciardelli TL.**

Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire 03755.

The baculovirus-mediated eukaryotic insect cell expression system was used to prepare large quantities of the beta-subunit ectodomain of the high-affinity interleukin-2 receptor (IL-2R beta x). We describe the expression, purification, and biophysical characterization of this ligand binding domain. The human cDNA encoding IL-2R beta x was inserted into baculovirus transfer vectors. High titer recombinant baculovirus was produced in *Spodoptera frugiperda* (Sf9) insect cells, and the viral supernatants were subsequently used to infect monolayers of *Trichoplusia ni* (High Five) insect cells in serum-free culture. Maximal expression of the recombinant protein excreted into the cell culture supernatants was determined by SDS/PAGE analysis, where a band migrating with an apparent molecular mass of 31 kDa was identified by immunostaining. One-step purification was achieved by affinity chromatography on either a monoclonal antibody (TIC-1) column or an IL-2 column, with a final yield of approximately 5 mg/L of culture supernatant. Interestingly, partial purification was also demonstrated using metal chelate affinity chromatography. Amino-terminal sequence analysis of the protein matched the published sequence. Both equilibrium sedimentation analysis and gel filtration chromatography indicated that IL-2R beta x remains monomeric. Deconvolution of far-UV circular dichroism (CD) spectra indicated the predominant secondary structural element to be beta-sheet, consistent with structural analysis and predictions for other members of the hematopoietic receptor family. A dissociation constant (K<sub>d</sub>) for IL-2R beta x in solution of 5.3 x 10<sup>-7</sup> M was calculated from competitive receptor binding assays. (ABSTRACT TRUNCATED AT 250 WORDS)

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